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Jayoti Vidyapeeth Women's University, Jaipur

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EFFECTS OF COW DUNG AND ITS ZINC NANOPARTICLES ON GERMINATION, GROWTH, BIOCHEMICAL AND YIELD PARAMETERS IN TOMATO AND OKRA

Submitted by

PAWAN KUMAR KANSOTIYA

Enrollment No :

JVR-l/22/10097

Under the Guidance of

DR. Y. CHANDRAKALA

Department of Science & Technology

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A Synopsis

Proposal Submitted in

fulfillment of the requirements for the award of the Degree of

DOCTOR OF PHILOSOPHY

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1. Introduction

Ensuring food security in developing countries is highly challenging due to low productivity of the agriculture sector, degradation of natural resources, high post farming losses, less or no value addition, and high population growth. Researchers are striving to adopt newer technologies to enhance supply to narrow the food demand gap. Nanotechnology is one of the promising technologies that could improve agricultural productivity via nano fertilizers, use of efficient herbicides and pesticides, soil feature regulation, wastewater management, and pathogen detection. Nanotechnology can also reduce post-farming losses by increasing the shelf life with the aid of nanoparticles. However, further investigation is required to solve the safety and health risks associated with the technology (Neme *et al*., 2021).

Cow dung, an excreta of bovine animal, is a cheap and easily available bioresource on our planet (Gupta *et al*., 2016). Cow dung can be defined as the undigested residue of consumed food material being excreted by herbivorous bovine animal species. Being a mixture of faeces and urine in the ratio of 3:1, it mainly consists of lignin, cellulose and hemicelluloses. It also contains 24 different minerals like nitrogen, potassium, along with trace amount of sulphur, iron, magnesium, copper, cobalt and manganese. The indigenous Indian cow also contain higher amount of calcium, phosphorus, zinc and copper than the cross-breed cow (Garg and Mudgal 2007; Randhawa and Kullar 2011). Cow dung harbours a rich microbial diversity, containing different species of bacteria (*Bacillus* spp., *Corynebacterium* spp. and *Lactobacillus* spp.), protozoa and yeast (*Saccharomyces* and *Candida*) (Nene 1999; Randhawa and Kullar 2011). Sawant *et al*. (2007) have isolated many different bacterial genera such as *Citrobacter koseri*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Kluyvera* spp., *Morgarella morganii*, *Pasteurella* spp., *Providencia alcaligenes*, *Providencia stuartii* and *Pseudomonas* spp. from cow dung.

In India, 69.9 % population resides in rural areas (The Hindu 2011), where cow (*Bos indicus*) is major cattle and generates 9–15 kg dung/day (Brown 2003). Waste is generally meant for discarding because it may act as a source of pollution (Pongrácz and Pohjola 2004). However, if it is used in some other process such as feedstock it may be considered as co-product (Brown 2003).

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Chemical fertilizers are instrumental in improving the crop productivity, but their excess and unguarded use damaged the food quality and soil health[6,7,8]. The use of cattle manure, or cow dung, at small scale agriculture and garden is a popular & advantageous practice which the farmers use in many rural areas to make their soil fertile enough. Generally, this type of manure is not as rich in nitrogen as compared to chemical fertilizers; however, the plants may get damaged due to the high ammonia levels when the fresh manure is directly applied. If this raw cow dung is once composted, it can provide numerous benefits to the garden (Atulesh, 2011).

Now a days, the applications of chemical and engineered nanoparticles (ENP) as fertilizers and for pest management have been increasing every year. To overcome the ecotoxicological impact of the chemical fertilizers and ENPs in soil system, researchers are trying to utilize biological and greener approach for the synthesis of nanoparticles. The biological extract from different plants and microbial sources utilized in the biosynthesis are safe, cost effective and having natural capping and reducing agents and phytochemicals like polyphenol, proteins and ascorbic acid. Therefore, the scientists are exploring various plant extract, flowering, fruiting parts, microbial and cellular components, etc., for the green synthesis of the nanoparticles (Senthamarai and malaikozhundan, 2022).

Zn^{+2} is an essential trace element for the metabolic process that occurs in plants as well as humans. The ZnO nanoparticles are considerably less toxic and declared as safe by FDA. Now a days, the zinc oxide nanoparticles are gaining a lot of attention due to the nontoxic behavior and higher requirement of the plants growth and development as well as the deficiency of Zn in soil (Itroutwar *et al*., 2020). Many researchers have used zinc oxide nanoparticles as a foliar fertilizer because they increase the agro morphological traits, photosynthesis, biomass, and yield of the plants (Munir, 2018).

Vegetables are parts of plants that are consumed by humans or other animals as food. The original meaning is still commonly used and is applied to plants collectively to refer to all edible plant matter, including the flowers, fruits, stems, leaves, roots, and seeds. An alternative definition of the term is applied somewhat arbitrarily, often by culinary and cultural tradition. Vegetables can be eaten either raw or cooked and play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals and dietary fiber. Many nutritionists encourage people to

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consume plenty of fruit and vegetables, five or more portions a day often being recommended. Both Tomato and Okra are common vegetables consumed in India and worldwide.

2. Objectives

The proposed investigation will be carried out to fulfil following objectives-

1. Synthesis and characterization of zinc oxide and copper nanoparticles using cow dung.
2. Evaluation of their effects on seed germination parameters of tomato and okra and compared to Zn, Cu and cow dung application.
3. Evaluation of their effects on morphological, biochemical and yield parameters on tomato and okra crops in comparison to Zn, Cu and cow dung.

3. Research gap

Zinc and copper nanoparticles synthesized by green methods have shown positive effects on various crops including tomato and okra also. Effects of cow dung manure have also been observed on these plants previously. A researcher also synthesized zinc oxide nanoparticles using cow dung extract and applied it on Mungbean. However, no study was reported synthesis of copper oxide nanoparticles using cow dung and their applications in agricultural sector. Also, there is no study related to application of ZnONPs synthesized using cow dung on the selected vegetable crops (tomato and okra). Any study was not found to evaluate effects on these nanoparticles on the quality of fruits/vegetables (Mineral and other nutritional parameters). The proposed study will be done first time ever.

4. Methodology

Synthesis of ZnO nanoparticles

To synthesize ZnO Nanoparticles, 90 ml of 0.02 M zinc nitrate hexahydrate solution will be prepared and heated at 30 °C ± 2 °C for about 10 min. Cow dung extract will then slowly add while continuous stirring. The solution will then kept for 2 h to settle precipitate at the bottom. The solution will then centrifuged for 10 min. Pellet will be collected and washed with deionized water. The formation of a creamy precipitate will

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indicate the presence of zinc oxide nanoparticles and resulting product was dried overnight at in hot air oven (Javed *et al*., 2022).

Synthesis of Copper nanoparticles

The procedure will be similar to ZnO. Instead of zinc nitrate Copper (II) Sulfate pentahydrate ($CuSO_4.5H_2O$) will be sued as Cu source.

Characterization of the synthesized green nanoparticles

The synthesized nanoparticles will be characterized for their size, shape, purity by –

- UV
- FTIR
- FESEM
- EDX
- XRD

Evaluation of effects of nanoparticles on seed germination parameters

Seeds of tomato and okra will be surface sterilized and soaked in different concentrations of the prepared nanoparticles (concentration will be decided later) and those were kept on filtered papers in petri dishes separately for germination. Besides these nanoparticles, seeds treated with Cu/Zn and cowdung alone will also be kept for germination along with control (water only).

The seed germination percentage, number of days to seed germination, total seedling length, shoot length, root length, fresh weight, dry weight will be measured.

After seed germination test, the best concentration range for germination will be decided for pot experiment.

Pot experiment

Characterized soil will be used for the experiment. Seeds (tomato and okra) will be soaked in soil and treatment will be given for different concentrations (3 concentrations) of Cu, Zn, Cowdung, ZnO/cowdung nanoparticle and Co/cowdung nanoparticles along with control (water only).

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Determination of Morphological parameters

Plant height, total number of branches/ plant, total number of leaves/branch, days to flowering, number of flowers at different days etc.

Yield parameters

Number of fruits per plant at different days, fruit weight/plant.

Biochemical parameters

Determination of total flavonoid content (Kaviarasan *et al*., 2007), total phenolic content (Ghasemzadeh *et al*., 2010), total carbohydrates (Hedge and Hofreiter, 1962), total proteins (Lowry *et al*. 1957), total lipids (Folch *et al*. (1957).), fibers ((Raghuramulu *et al*., 2003), minerals (Raghuramulu *et al*., 2003) etc. will be determined in fruits (as these crops are grown for their fruits mainly) using their standard methods.

Note*: all the experiments will be conducted in triplicates and statistical analysis will be done to know the presence/absence of significance variations among various treatments.

5. Major Hypothesis

The salts for nanoparticles will be purchased from standard company. Cow dung will also be collected using good hygienic practices. Nanoparticles will be synthesized using quality standards. Characterization will be done at laboratory recognized by Indian Standards. During all experiments either petri plates/ pot culture/ biochemical evaluation, all standards will be followed.

6. Year-wise work plan

First year:

- Synthesis and characterization of both kinds of nanoparticles.
- Petri plate experiment and to evaluate the effects of nanoparticles on seed germination parameters.

Second year

- Pot experiment for both crops
- Morphological parameters observation
- Yield parameters observation
- Collection and processing of fruits for biochemical assay.

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Third year work plan

- Calculations and statistical analysis of the observed data

Remaining tenure will be used for writing research papers and thesis.

7. Instrumentation used

For the whole study, following instruments will be utilized-

- Weighing balance
- Magnetic stirrer
- Sonicator
- Hot air oven
- Hot plate
- Incubating shaker
- UV spectrophotometer
- FTIR
- FESEM
- Centrifuge
- Laminar air flow
- Autoclave etc.

8. Details of External Resources

For characterization of the synthesized nanoparticles, facilities of MRC, MNIT, Jaipur, and Biomitra Life Sciences Pvt. Ltd., Jaipur will be utilized.

9. Significance of the proposed study

If the results of the proposed study will be in positive way then it will help farmers a lot. There will be dual benefits in form of waste management (cow dung management) as well as crop improvement.

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